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ROG1 encodes a monoacylglycerol lipase in *Saccharomyces cerevisiae*

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ABSTRACT

Lipid metabolism is extensively studied in *Saccharomyces cerevisiae*. Here, we report that revertant of glycogen synthase kinase mutation-1 (Rog1p) possesses monoacylglycerol (MAG) lipase activity in *S. cerevisiae*. The lipase activity of Rog1p was confirmed in two ways: through analysis of a strain with a double deletion of *ROG1* and monoglyceride lipase *YJU3* (*yju3*Δ*rog1*Δ) and by site-directed mutagenesis of the *ROG1* lipase motif (GXSXG). Rog1p is localized in both the cytosol and the nucleus. Overexpression of *ROG1* in a *ROG1*-deficient strain resulted in an accumulation of reactive oxygen species. These results suggest that Rog1p is a MAG lipase that regulates lipid homeostasis.

1. Introduction

Lipids are biological macromolecules that are essential for cell survival. *Saccharomyces cerevisiae* is an excellent model system to study lipid metabolism and homeostasis in a controlled manner [1]. Recently, many yeast genes involved in lipid metabolism have been characterized and their physiological functions elucidated [2,3] by comparison with their counterparts in other organisms, such as humans, *Arabidopsis thaliana*, *Drosophila melanogaster*, *Caenorhabditis elegans* [4–6]. Some proteins have been reported to have multiple functions that are apparently distinct in their activities, such as lipase and acyltransferase functions [7,8]. Many proteins were first characterized as having a particular function, but later identified to have an additional function [9]. It is probable that the budding yeast gene YGL144c also belongs to this category. It was previously annotated as a Revertant of Glycogen synthase kinase mutation 1 (*ROG1*) [10], and sequence analysis in the *Saccharomyces* Genome Database (SGD) identified the presence of a putative lipase motif GXSXG, suggesting possible lipase activity.

Triacylglycerol (TAG) and sterol esters (SE) are stored in the lipid droplets [11] and become mobilized by lipases whenever there is an energy demand. In yeast, many lipases (*TGL3*, *TGL4*, *TGL5* and *AYR1*) have been reported to specifically degrade TAG [12,13,2]. *TGL2* hydrolyzes both TAG and diacylglycerol (DAG) in mitochondria [14]. However, for monoacylglycerol (MAG) hydrolysis, Yju3p is the only known reported MAG lipase in yeast, hydrolyzing MAG to fatty acid and glycerol [15]. In SGD, many genes are as yet uncharacterized, and some have putative lipase motifs. MAG has a role in regulating various cellular functions and is involved in signaling, insulin secretion and brain functions of higher organisms [16]. MAG lipase has been found to increase tumor aggression by regulating the fatty acid network. In addition to being enriched in oncogenic signaling, lipids promote migration, invasion, survival and in vivo tumor growth. Daum et al. [17] have reported that the systemic deletion of the ORF for YGL144c (*ROG1*) significantly affected lipid levels. With this lead, we proceeded to further elucidate the role of *ROG1* in lipid metabolism. *ROG1* has a putative serine active lipase domain, and the protein has a molecular weight of 78.12 kDa and consists of 685 amino acids. Based on the sequence similarity search and domain search in the National Center for Biotechnology Information and SGD databases, the protein has the lipase motif (G267XSXG271). We cloned *ROG1* in a yeast expression system and transformed it in wild type (BY4741), *rog1*Δ, *yju3*Δ and double deletion (*yju3*Δ *rog1*Δ) strains.

Abbreviations: SGD, Saccharomyces Genome Database; NCBI, National Center for Biotechnology Information; MAG, monoacylglycerol; ORF, open reading frame

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Here, we report thatRog1p is a MAG lipase and hydrolyzes monoolein to oleic acid and glycerol. Andoh et al. [10] hypothesized that *ROG1* could influence the mitochondrial functions, and accordingly, we observed reactive oxygen species (ROS) using fluorescence microscopy. Further in silico analyses revealed that Rog1p interacts with MAG (data not shown). In the current study, Rog1 is shown to encode MAG lipase.

2. Materials and methods

2.1. Chemicals and media

Yeast extract, peptone, Yeast Nitrogenous base and bacteriological agar were purchased from Difco. [1-^{14}C]Mono-oleoyl-*rac*-glycerol (55 mCi/mmol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). Thin-layer silica gel 60 plates were purchased from Merck. BODIPY™ 493/503 stain for lipid droplets was purchased from Invitrogen. Dodecyl maltoside (DDM), phenylmethylsulfonyl fluoride (PMSF), monoclonal anti-His-(6×) antibody and all other chemicals were purchased from Sigma. Lipids and lipid standards were obtained from Avanti Polar Lipids (Alabaster, AL).

2.2. Plasmids, strains and culture conditions

The plasmids used in this study were pYES2/NTC (poly-His (6×) tag), pUG34, pYES2/NTC-*ROG1*, pUG34-*ROG1*. The wild-type *S. cerevisiae* strain (BY4741-MATa; *his3Δ1*; *leu2Δ0*; *met15Δ0*; *ura3Δ0*), *rog1*Δ and *yju3*Δ strains on the BY4741 background were obtained from Euroscarf. Cultures were maintained in YPD (1% yeast extract, 2% peptone, 2% dextrose) medium. Protein expression was induced by growing the cells aerobically at 30 °C in synthetic medium without uracil (SM-U) in the presence of 2% galactose for 24 h.

2.3. Construction of the overexpression plasmid and GFP tag

Yeast genomic DNA was isolated from the wild-type cells and used as a template for PCR amplification of *ROG1* using a standard protocol. The forward primer was 5′-ATAAGAATTCATGTCTCTGAC ACCAACTAAT-3′ and the reverse primer was 5′-ATAAGAGCTCTCAT TGTACCAAATCACTATTC-3′. The following conditions were maintained for amplification of *ROG1* in an Eppendorf thermal cycler: initial denaturation of the template at 94 °C for 3 min followed by 26 cycles of 30 s of denaturation at 94 °C, 30 s of annealing at 57 °C and 1.5 min of elongation at 72 °C. The final extension was carried out at 72 °C for 10 min. The purified PCR product and the vector pYES2/NTC were digested with EcoRI and XhoI, ligated directionally and then transformed into *Escherichia coli* DH5α cells. The resultant positive clones were confirmed by double digestion and nucleotide sequencing. For the construction of the *ROG1*-GFP plasmid, the *ROG1* gene and pUG34 were double digested using EcoRI and XhoI, ligated and then transformed into *E. coli* DH5α cells. The resultant positive clones were confirmed by double digestion. The constructs pYES2/NTC-*ROG1* and pUG34-*ROG1* were transformed into yeast cells using the standard lithium acetate protocol.

2.4. Purification of His-tagged Rog1p

Yeast cells overexpressing Rog1p were grown in synthetic media without uracil containing 2% glucose for 24 h, and the cells were harvested and induced with 2% galactose for 24 h. Cells were harvested and suspended in lysis buffer containing 50 mM Tris–HCl pH 8.0, 1 mM $MgCl_2$, 1 mM EDTA, 10% glycerol and 1 mM PMSF. Cells were lysed using glass beads and unbroken cells were removed by centrifugation at 5000×*g* for 10 min. The cell-free extract was centrifuged at 30000×*g* for 30 min to obtain the membrane and the soluble fractions. The membrane fraction was solubilized with 10 mM DDM in lysis buffer and incubated at 4 °C for 60 min and centrifuged at 30000×*g* for 60 min. The solubilized membrane fraction and cytosolic fraction were then loaded onto a Ni^{2+}-NTA column and the protein was eluted according to the manufacturer's instructions. The purified protein in the eluted fractions was analyzed by 12% SDS–PAGE followed by Coomassie brilliant blue staining. Protein expression was confirmed by immunoblotting using a monoclonal anti-His antibody produced in mouse at a dilution of 1:5000 (v/v). The secondary rabbit anti-mouse IgG polyclonal antibody was tagged with alkaline phosphatase and detected using the BCIP/NBT assay. Protein concentrations were determined using the protein–dye binding assay with bovine serum albumin as a standard.

2.5. In vitro MAG lipase assay

[^{14}C]MAG was used as a substrate to assay the MAG lipase activity of the purified ROG1 purified protein from the membrane and cytosolic fractions. The assay mixture consisted of 50 mM Tris–HCl pH 8.0, 1 mM DTT, 2 μg of dialyzed protein, 50 μM [^{14}C]MAG (0.025 μCi) in a final volume of 100 μl. The reaction was carried out at 30 °C for 30 min and stopped by the addition of chloroform:methanol (2:1). The lipids were then extracted and resolved on silica-TLC plates using petroleum ether:diethyl ether:glacial acetic acid (70:30:1, v/v) as the solvent system. The plates were developed using the Phosphor imaging system (Amersham Biosystems) and the spots corresponding to MAG and free fatty acids were quantified using a liquid scintillation counter.

2.6. Construction of the double mutant

To create a double deletion strain of *yju3*Δ*rog1*Δ, *yju3*Δ on a wild type background was chosen. The yEP351 leucine cassette was chosen and primers were designed with regard to the in frame positioning of the leucine gene and flanking to the corresponding ROG1 position in the open reading frame (ORF). The primers used were: forward primer 5′CAGTAAGAATAGTACACAAAGAGAGTGAA GTACGAACCGTGTTTTGCTCAAGATGACTGTGGGAATACTCAGGT 3′ and reverse primer 5′ AAATGTAACTCCA-GACCATTCATAAACCGT AAATAAATATATAAAGCTCTATCACAAATTAGGGATTCGTAGTT3′. The insert was amplified using the primers, purified and transformed into yeast *yju3*Δ cells in SM-L selection media. The colonies were screened for double deletion using the screening primers (forward primer 5′-CTGACGCACATAGTAGATGCCCGAAATA-3′ and reverse primer 5′-CCAAATGCGGTGTTCTTGTCTGGCAAAGAG-3′) to ensure homologous recombination of the leucine gene in the *ROG1* position.

2.7. MAG lipase activity in cell lysates

Wild type, single deletion mutants of *YJU3* and *ROG1* and double deletion mutant (*YJU3 ROG1*) yeast were grown in synthetic complete medium for 24 h and the cell lysates were prepared using lysis buffer (50 mM Tris–HCl pH 8.0, 1.0 mM $MgCl_2$, 1.0 mM EDTA, 10% glycerol) containing 1.0 mM PMSF. Cell lysates (5 μg protein) were used for the MAG lipase assay. The assay mixture consisted of 50 mM Tris–HCl pH 8.0, 1 mM DTT, 5 μg of dialyzed protein, 50 μM MAG in a final volume of 100 μl. The reaction was carried out at 30 °C for 10 min and stopped by the addition of chloroform:methanol (2:1), the lipids were extracted. The lipids were resolved on silica-TLC plates using petroleum ether:diethyl ether:glacial acetic acid (70:30:1, v/v) as the solvent system and the plates were developed in the iodine chamber. The spots

corresponding to MAG and free fatty acids were quantified using densitometry. The Student's *t*-test analysis was performed for *yju3*Δ and *yju3*Δ*rog1*Δ using Graph Pad Prism software.

2.8. Site-directed mutagenesis

Mutations in the G*X*S*X*G motif (G267A, S269A, G271A) were achieved using the following primers: forward primer was 5′-AAGATTTCTTTTGTAGCTCATGCATTGGCTGGCCTAATCCAGGCT-3′, and reverse primer was 5′-AGCCTGGATTAGGCCAGCCAATGCAT-GAGCTACAAAAGAAATCTT-3′. The underlined base pairs indicated the sites of mutation. The reaction mixture contained wild-type pYES2/NTC–*ROG1* template (100 ng), primers (125 ng each), 0.2 mM dNTPs, reaction buffer and enzymes. Amplification was performed using the following conditions: denaturation of the template at 95 °C for 2 min followed by 18 cycles at 95 °C for 20 s (denaturation), 60 °C for 10 s (annealing) and 68 °C for 30 s (extension). The final extension was carried out at 68 °C for 5 min. After amplification, the reaction product was incubated with DpnI at 37 °C for 1 h to digest the non-mutated plasmid and transformed into *E. coli* DH5α cells. Mutation of the gene was confirmed by sequencing. The purified recombinant mutant proteins were used in the enzymatic assays.

2.9. Lipid droplets staining

The yeast strains *rog1*Δ and *yju3*Δ*rog1*Δ were transformed with pYES2/NTC and the cells were grown for 24 h in synthetic medium containing 2% (w/v) glucose. Then, 0.4 OD of cells were induced with 2% (w/v) galactose and grown for 24 h in the synthetic medium with appropriate selection markers (SM-U and SM-UL). An equal amount (10 OD) of cells treated with BODIPY for 30 min in the dark and washed twice with phosphate-buffered saline (PBS) and viewed under a confocal microscope to visualize the lipid droplets (100 × oil objective on a Zeiss LSM710 microscope).

2.10. Localization of Rog1p by confocal microscopy

Yeast cells containing *ROG1*-GFP in the pUG34 vector and wild-type cells with *ROG1*-GFP integrated in the chromosome and a constitutively expressed cytosolic mCherry were grown in SM-H medium for 24 h and then treated with DAPI (1 μg ml^{-1}) for 30 min and washed with PBS. Then, the fluorescent cells were visualized by confocal microscopy using a 100 × oil objective on a Zeiss LSM710 microscope.

2.11. ROS staining by fluorescent microscopy

The yeast strains (wild-type, *rog1*Δ, *yju3*Δ and the double deletion *yju3*Δ*rog1*Δ) were grown in 2% (w/v) glucose in the SM-U medium for 24 h and 0.4 OD cells were inoculated. For induction, 2% (w/v) galactose was used in the SM-U medium and the cells were harvested after 24 h, treated with 10 μM of 2,7-dichlorofluorescein diacetate (DCF) for 30 min, washed thoroughly with PBS and observed using fluorescent microscopy.

3. Results

3.1. ROG1 encodes a MAG lipase

ROG1 was cloned into the pYES2/NTC vector, and the protein was overexpressed in the *yju3*Δ strain on the BY4741 background. The membrane fraction was solubilized with DDM and purified using a Ni–NTA column, and the eluted protein was loaded onto a 12% SDS–PAGE and visualized by silver staining (Fig. 1A) and immunoblot analyses (Fig. 1B). The purified protein had a molecular weight of ~82 kDa. To determine if Rog1p had lipase activity, [^{14}C] labeled MAG, DAG and TAG were used to determine the activity and specificity of the purified Rog1p. Rog1p hydrolyzed MAG alone, and did not hydrolyze DAG and TAG (data not shown). Purified Rog1p from the soluble (Fig. 1C) and membrane fractions (Fig. 1D) was used for the assay and the release of free fatty acid from MAG was observed. Phospholipase assays were also performed for Rog1p using phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, phosphatidylglycerol and phosphatidic acid in the presence of Ca^{2+}, but the purified recombinant protein did not show any detectable activity towards these lipids (data not shown). These data suggested that Rog1p is a lipase with the preference for MAG.

3.2. Characterization of Rog1p MAG lipase activity

The *ROG1* gene was overexpressed, and the protein was purified from the membrane fractions. The enzyme activity was initially tested using 2 μg of purified Rog1p and 50 μM [^{14}C]MAG for 10 min at 30 °C (Fig. 2). MAG lipase activity was also studied using the following parameters: varying temperatures ranging from 0 to 55 °C (Fig. 2A); varying pH from 4 to 10 (Fig. 2B); various MAG concentrations (Fig. 2C and D); increasing amounts of protein (Fig. 2E) and at different time intervals (Fig. 2F). In addition, the preference

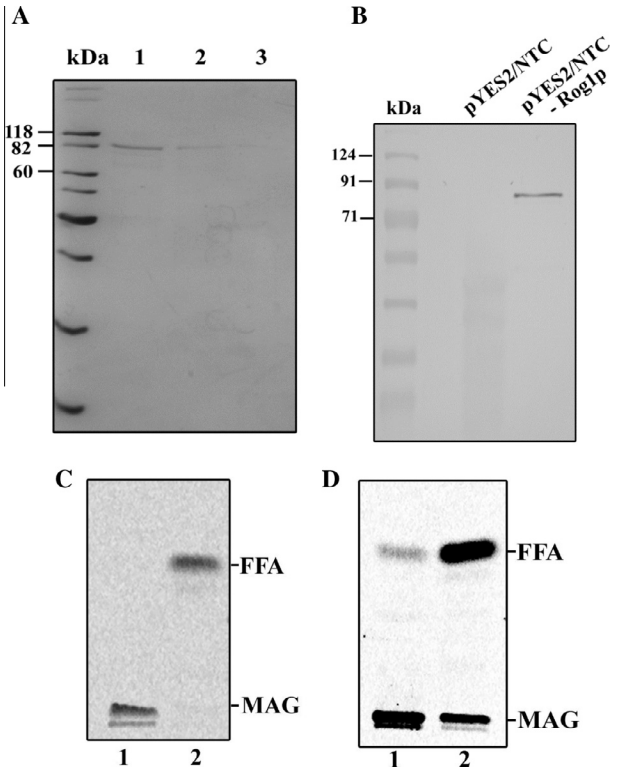

Fig. 1. *ROG1* encodes a MAG lipase. pYES2/NTC-*ROG1* was transformed into the *yju3*Δ strain in BY4741 and induced with galactose for overexpression. The protein from the membrane fraction was solubilized using DDM and purified by Ni–NTA column chromatography. (A) The eluted proteins were resolved by 12% SDS–PAGE followed silver staining. *kDa – Protein molecular mass Marker, 1, 2 and 3 are the eluted fractions*. (B) The expression was analyzed by Western blot analysis using an anti-His monoclonal antibody. *pYES2/NTC was the empty vector and the pYES2/NTC-ROG1 was used to overexpress ROG1 in the yju3*Δ *strain*. (C) The purified recombinant protein (2 μg) from the cytosol and the membrane solubilized (D) fractions were used in enzyme activity assays (*1 – substrate alone without enzyme; 2 – purified Rog1p along with substrate*).

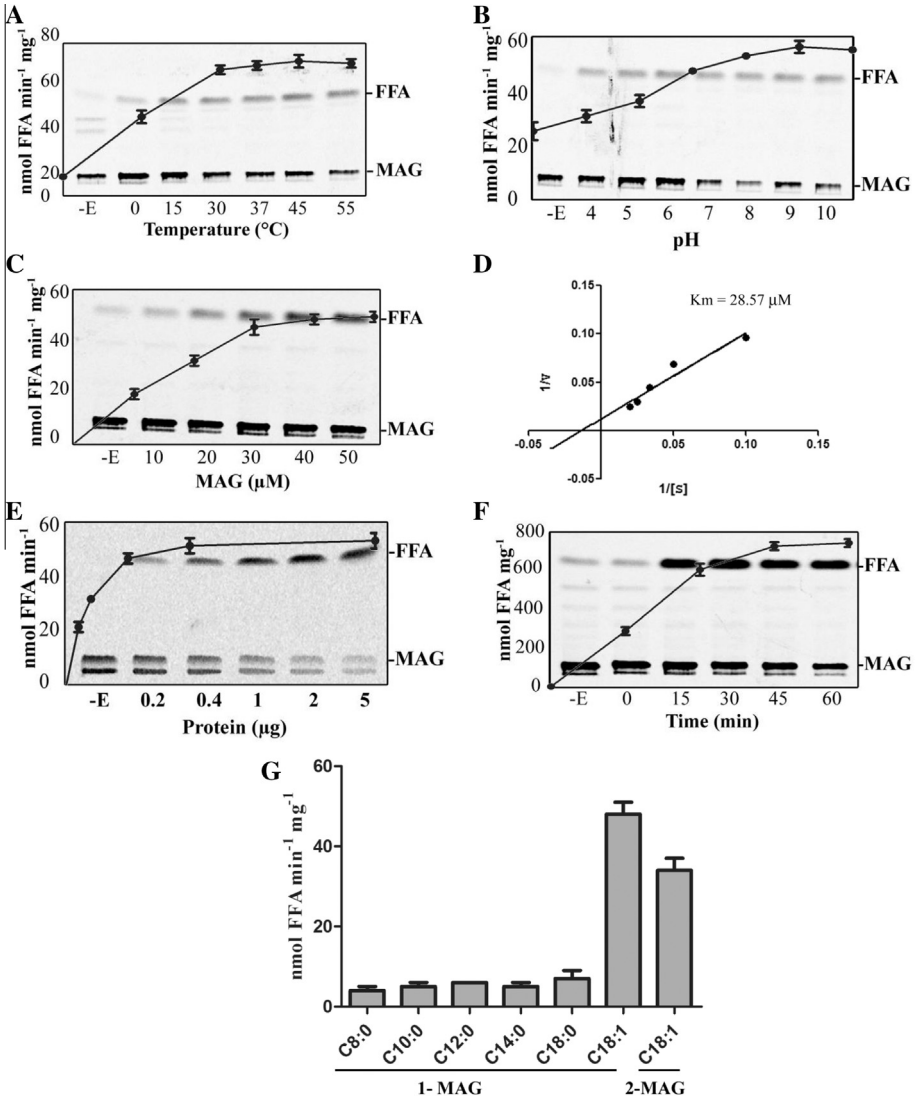

Fig. 2. Characterization of Rog1p MAG lipase activity. (A) MAG lipase assays were conducted at various temperatures, ranging from 0° to 55 °C, (B) increases in pH from 4 to 10 using different buffers, such as 50 mM citrate (pH range 4–6), 50 mM Tris–HCl (pH 7 and 8) and 20 mM glycine-NaOH (pH 9 and 10), (C and D) increases in substrate concentration from 0 to 50 µM, (E) increases in protein concentration from 0.2 to 5 µg and (F) increases in incubation time from 0 to 60 min. MAG with varying fatty acids (C8:0 to C18:1) was assayed to identify the preferred substrate (G). The reaction was stopped using chloroform: methanol (2:1) and the lipids were extracted and separated by TLC and developed in the phosphorimager. The data represent the mean ± S.D. of three independent experiments conducted in duplicate, with statistical significance set at $^{*}P < 0.05$.

for the fatty acid chain lengths in MAG substrate was determined [1-MAG (C8:0, C10:0, C12:0, C14:0, C18:0, C18:1) and 2-MAG] and we found that the MAG (oleoyl) was a preferred substrate compared to other MAG counterparts (Fig. 2G). MAG lipase was found to be active at broad temperature (15–55 °C) and pH (4–10) ranges. The enzyme had the maximal activity with 50 µM MAG and 2 µg protein with a km of 28.57 µM.

3.3. The double mutant (*yju3Δrog1Δ*) has reduced MAG lipase activity

YJU3 is the only reported MAG lipase in yeast (15). Therefore, a double mutant (*yju3Δrog1Δ*) was generated to remove both the Yju3p and Rog1p MAG lipase activities in yeast. To determine if the MAG lipase of Rog1p contributes significantly in yeast, the MAG lipase activity assay was performed in wild-type, *rog1Δ*, *yju3Δ* and *yju3Δrog1Δ* cell lysates. The maximum reduction in MAG lipase activity was observed in *yju3Δ rog1Δ* compared to other strains (Fig. 3; *yju3Δ* and *yju3Δrog1Δ* = 1.387, *P* value of 0.238, and 95% confidence interval from −3.342 to 10.007). The residual activity in the *yju3Δrog1Δ* strain could have been from unannotated lipases.

3.4. The lipase motif is important for enzyme activity

The importance of the lipase motif (GXSXG) was assessed by site-directed mutagenesis. Equal amounts of protein from Rog1p and lipase motif mutated Rog1p were analyzed by western blotting using a monoclonal anti-His antibody. The site-directed mutation did not affect protein expression (Fig. 4A). However, the MAG lipase activity was drastically decreased (∼95%) when all three amino acids at the catalytic site were mutated (Fig. 4B and C).

3.5. Lipid droplet accumulation in *yju3Δ rog1Δ* cells

The accumulation of lipid droplets (LDs) in *rog1Δ* and *yju3Δrog1Δ* were analyzed by staining the cells with BODIPY™493/503. The LDs of *yju3Δrog1Δ* were of higher number and larger size (Fig. 5) compared to *rog1Δ*. The deletion of Rog1p leads to

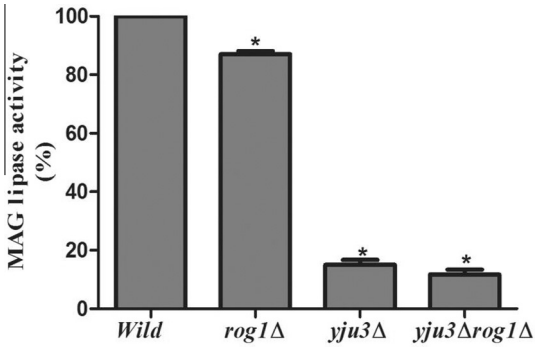

Fig. 3. MAG lipase activity in whole cell lysates wild-type, single deletions of *yju3* and *rog1* and double deletion of *yju3 rog1* yeast strains were grown in a synthetic complete medium for 24 h. The cells were harvested and lysed with glass beads. The crude lysates (5 μg) were used for the lipase assay with 50 μM MAG as a substrate. The reaction mixture was incubated for 10 min at 30 °C. The reaction was stopped using chloroform: methanol (2:1) and the lipids were extracted and separated by silica-TLC followed by staining with iodine. The stained lipids were quantified by Quantity One Software. The data represent the mean ± S.D. of three independent experiments conducted in duplicate, wild-type cells were compared to the knock out groups, with statistical significance set at $^{*}P < 0.05$.

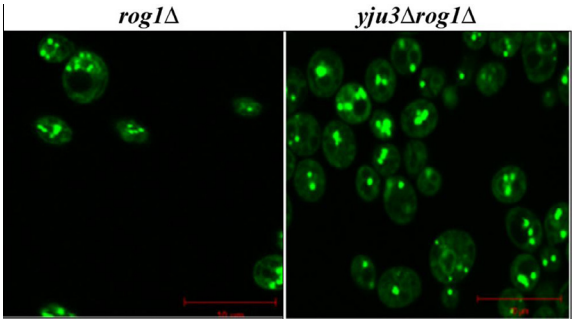

Fig. 5. Lipid droplet visualization. *rog1*Δ and *yju3*Δ *rog1*Δ strains were grown in glucose for 24 and then 10 OD cells were treated with BODIPY493/503 for 30 min, washed with PBS and visualized under a confocal microscope.

abnormal LD morphology which implied the involvement of Rog1p in LDs.

3.6. Rog1p is localized in the nucleus and cytosol

The physiological function of a protein is greatly influenced by the localization of the protein. Localization of Rog1p was studied using confocal microscopy. Rog1p-GFP was localized in both the nucleus and the cytosol (Fig. 6A and B).

3.7. Overexpression of Rog1p increased ROS accumulation

It was hypothesized that *ROG1* could influence mitochondrial function [10]. To determine if Rog1p has a role in accumulating ROS, the strains (wild-type, *rog1*Δ, *yju3*Δ and *yju3*Δ*rog1*Δ) containing empty vector (pYES2/NTC) and pYES2/NTC-*ROG1* were stained with DCF and viewed under a fluorescent microscope. Each of the strains containing empty vector showed less uptake of the dye, indicating less accumulation of ROS at the intracellular level. In contrast, the Rog1p overexpressing strains revealed more ROS accumulation compared to the vector control. Rog1p

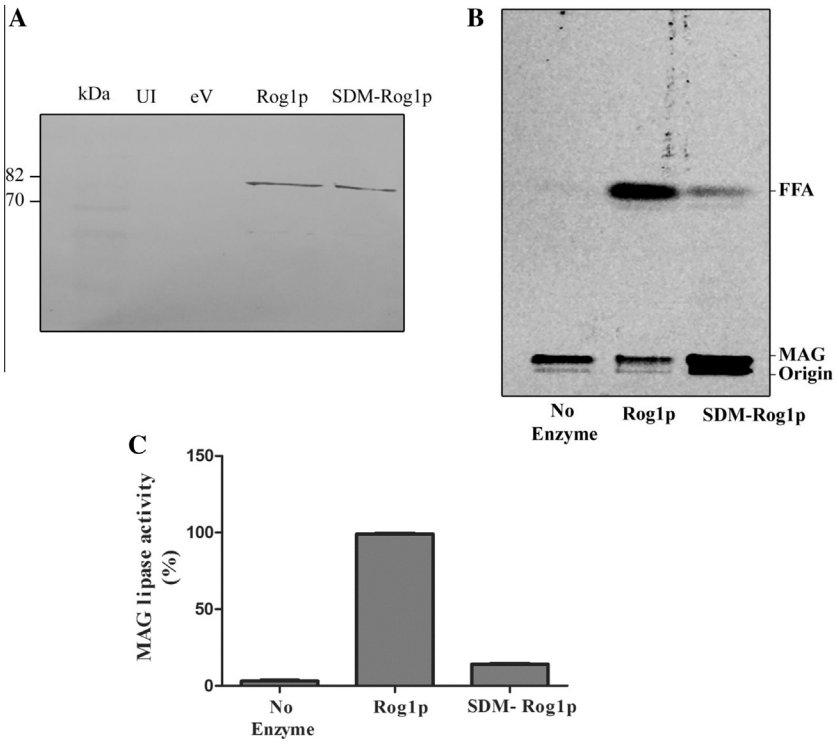

Fig. 4. Site-directed mutagenesis of the lipase domain. Specific forward and reverse primers were used for plasmid amplification to induce the site-directed mutation, and treated with DpnI (10 U) at 37 °C for 1 h to digest the methylated parent template. The newly amplified, unmethylated plasmid was then transformed into competent DH5α *E. coli*. The mutated plasmid was then transformed into the *yju3*Δ strain. The mutants were confirmed by sequencing the plasmid DNA using gene specific primers. Protein expression of Rog1p and mutated Rog1p is shown in (A). The MAG lipase assay was performed with the purified recombinant protein and mutated protein (B) and quantified in panel (C) (kDa – protein molecular mass marker, *UI – uninduced*; *eV – empty vector*; *SDM-Rog1p – Rog1p* $G_{267}A$, $S_{269}A$, $G_{271}A$). The data represent the mean ± S.D. of three independent experiments conducted in duplicate.

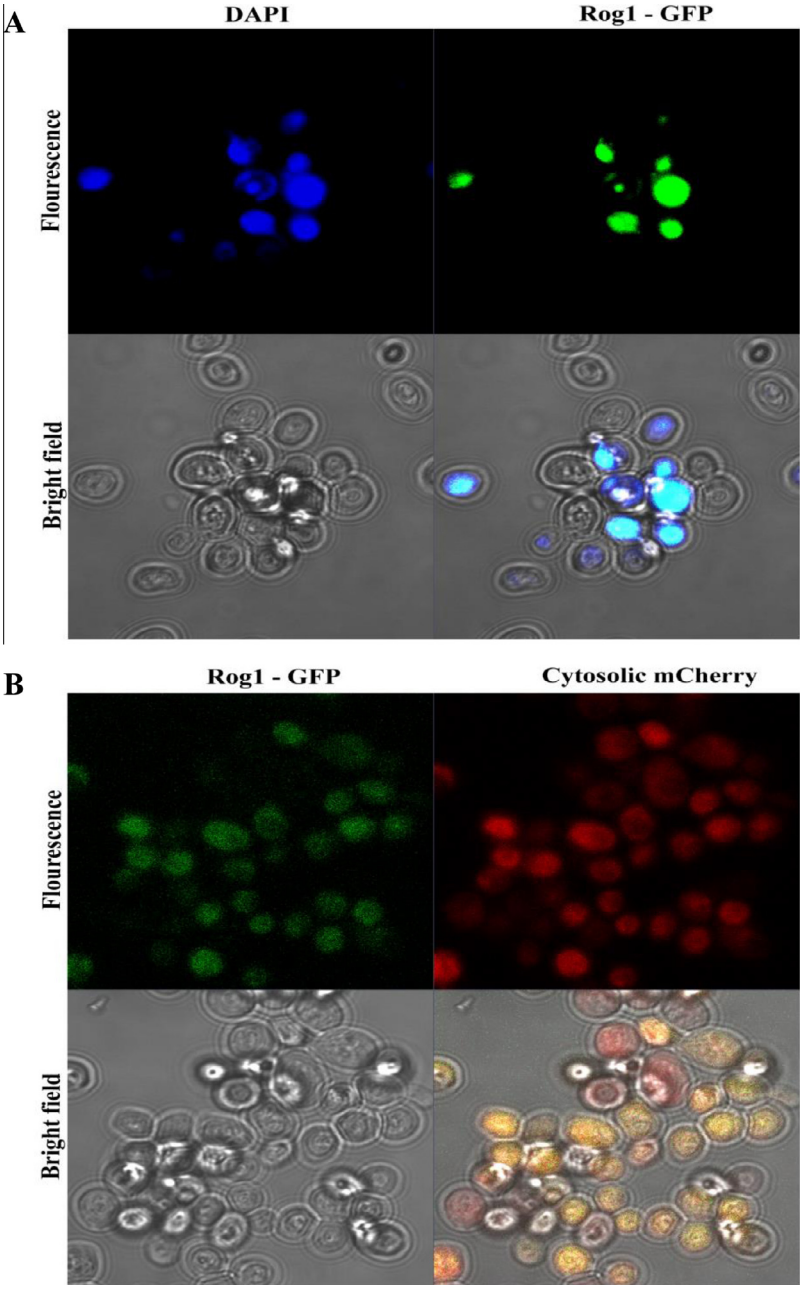

Fig. 6. Localization of Rog1p. *ROG1*-GFP was transformed into yeast cells. The cells were stained with DAPI for 30 min, washed and visualized under a confocal microscope (A). *ROG1*-GFP was transformed into yeast cells that constitutively express mCherry in the cytosol and observed under a confocal microscope (B).

overexpression in *yju3*Δ and *yju3*Δ*rog1*Δ showed a high level of ROS accumulation (Fig. 7).

4. Discussion

Lipases are essential hydrolytic enzymes in the cellular environment that maintain lipid homeostasis [18]. Rog1p was previously reported as revertant of glycogen synthase kinase 1, and has a putative lipase domain. Many lipases in yeast act on triacylglycerol [2,12,13], and only one enzyme, Yju3p, has been reported to possess MAG lipase activity [15]. Rog1p has the lipase catalytic domain and it belongs to the alpha/beta-hydrolase (ABH) family [SGD, 17]. ABH domain-containing proteins have lipid-related functions in the mammalian system [19]. To study its biochemical function, the *ROG1* gene was cloned, overexpressed (Fig. 1A and B), and the purified recombinant protein was used for enzyme studies. Both soluble and membrane fractions were tested for lipase activity. Both fractions showed MAG lipase activity (Fig. 1C and D). The purified recombinant protein was characterized for the effects of temperature, pH, substrate concentration, and enzyme concentration at different time points. Rog1p was found to be active at broad temperature (15–55 °C) and pH ranges (4–10), and had optimum functionality using 50 μM MAG and 2 μg protein (Fig. 2). The most preferred substrate was 1-oleoylglycerol. All of the assays pertaining to the enzyme characteristics were performed in the *yju3*Δ strain because Yju3p is the only reported enzyme with MAG lipase activity [15]. Hence, the experiments were carried out in the absence of *YJU3*. The Vmax for the Rog1p MAG lipase activity was 2.4 mmol min^{-1} mg^{-1}. The apparent km was calculated to be 28.57 μM compared to the km of Yju3p (260 μM), and mouse

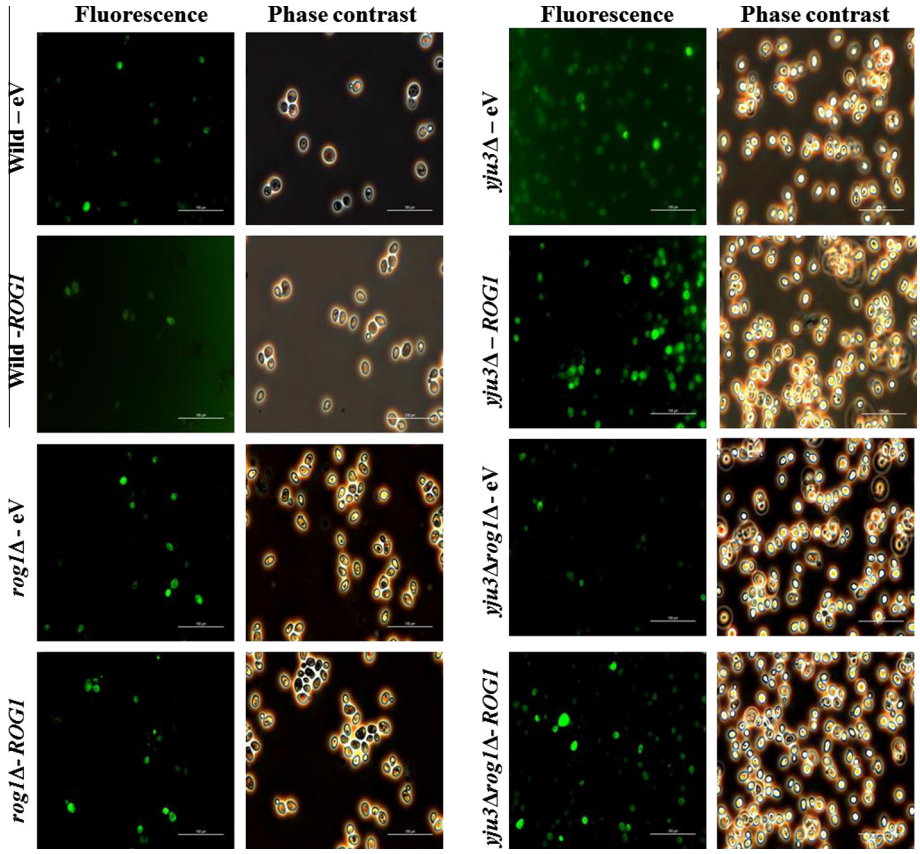

Fig. 7. Overexpression of Rog1p increases ROS accumulation. Yeast strains (wild-type, *rog1*Δ, *yju3*Δ and *yju3*Δ *rog1*Δ) containing empty vector and a *ROG1* overexpression vector were grown in 2% (w/v) galactose for 24 h and the cells treated with DCF for 30 min. The cells were washed with PBS and visualized under a fluorescent microscope.

(300 μM), and rat (200 μM) counterparts [15,20]. Further, the activity of MAG lipase was confirmed by *ROG1* overexpression in the *yju3*Δ*rog1*Δ double mutant (Fig. 4). These results suggest that Yju3p is the major MAG lipase and that Rog1p also has MAG lipase activity in yeast. The double deletion strain (*yju3*Δ*rog1*Δ) has residual MAG lipase activity, and the SGD shows many ORF having a lipase motif which may account for this residual activity; the products of these ORF are yet to be characterized.

The double deletion mutant *yju3*Δ*rog1*Δ showed a significant reduction in MAG lipase activity when compared with the single deletions, yju3Δ and rog1Δ (Fig. 3). The catalytic domain G*X*S*X*G is responsible for lipase activity. Site directed mutation in *ROG1* at the catalytic site (G267 to A267, S269 to A269 and G271 to A271; Fig. 4) showed greatly reduced MAG lipase activity when introduced into the *yju3*Δ strain. Mutation of the G*X*S*X*G motif usually abrogates the maximal lipase activity [3,13]. LDs were more prominent in *yju3*Δ*rog1*Δ cells compared to *rog1*Δ cells (Fig. 5). The localization of Rog1p was also studied using confocal microscopy. Rog1p has been reported to be present in the cytoplasm (SG database) and also predicted to be in the nucleus [21]. Rog1-GFP cells were stained with DAPI to identify nuclear localization (Fig. 6A), and the merging of GFP and DAPI signals indicated the presence of Rog1p in the nucleus and confirmed its presence in the cytoplasm through mCherry and GFP colocalization (Fig. 6B). Until now, nuclear lipids and enzymes involved in lipid metabolism have not gained much attention, but lipid droplets have recently been identified in the nucleus of rat hepatocytes [22], thereby providing a possible role for lipases (including MAG lipase) in mobilizing lipids. Rog1p was previously hypothesized to have a role in mitochondrial defects [10]. In the current study, ROS accumulation was observed in the wild-type, *rog1*Δ, *yju3*Δ and *yju3*Δ*rog1*Δ yeast strains overexpressing Rog1p that were treated with the ROS probe DCF (Fig. 7). *ROG1* reverts the mutation of glycogen synthase kinase (SG database), and, in the current study, increased ROS production was observed upon *ROG1* overexpression. Moreover, increased ROS production accelerates GSK3 activity [16], which regulates lipid homeostasis [23].

Conflict of interest

The authors declare that there is no conflict of interest.

Role of funding

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